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Acute and sub-chronic oral toxicity studies of an aqueous stem bark extract of *Pterocarpus soyauxii* Taub (Papilionaceae) in rodents

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ABSTRACT

Pterocarpus soyauxii Taub (Papilionaceae) is used in Cameroonian traditional medicine and pharmacopoeia to treat hypertension, diabetes, gastrointestinal parasitizes and cutaneous diseases.
Aim of the study: The present investigation was carried out to evaluate the safety of an aqueous stem bark extract of *Pterocarpus soyauxii* by determining toxicity after acute and sub-chronic oral administration in male and female rodents.
Materials and methods: The acute toxicity test was conducted in mice. An aqueous extract of barks was administrated by gavage in single doses of 2.5–12.5 g/kg. General behaviour and mortality were examined for up to 7 days. The sub-chronic toxicity test was performed in rats. The plant extract was administered by daily gavage of 150–600 mg/kg for 42 days. Body weight, food and water intakes were followed weekly. Haematological, biochemical and organ parameters were determined at the end of the 42-day administration.
Results: In the acute study in mice, oral administration of the aqueous extract of *Pterocarpus soyauxii* caused dose-dependent general behaviour adverse effects and mortality. The no-observed adverse effect level (NOAEL) of the extract was 5.0 g/kg. The lowest-observed adverse effect level (LOAEL) was 7.5 mg/kg. Mortality increased with the dose, LD_{50} was > 10.75 g/kg for the mouse. In the sub-chronic study in rats, daily oral administration of the aqueous extract of *Pterocarpus soyauxii* did not result in death or significant changes in haematological or biochemical parameters, excepted increased hepatic catalase activity ($P < 0.05$) at the dose of 600 mg/kg. No alteration was observed in body weight, food and water intake. Liver, kidney, lung and pancreas histopathology did not reveal morphological alteration.
Conclusions: The results showed that the aqueous stem bark extract of *Pterocarpus soyauxii* Taub had very low toxicity in oral acute high dose administration and no toxicity in oral sub-chronic low dose administration and indicate that the plant could be considered safe for oral medication.

1. Introduction

Several species of the genus *Pterocarpus* disseminated in tropical regions are widely used in Africa and Asia to treat diseases. *Pterocarpus soyauxii* is a 30–55-feet high rain forest tree. It belongs to the Fabaceae or Papilionaceae family, branch of spermaphytes (Burkill, 1995). The plant stem bark, grey-brown to brown-coloured, scales off in fine irregular scales and contains a red sap. Leaves, wood, stem bark, seed and flours are used in African traditional medicine, especially in the Cameroonian pharmacopoeia, for treating various diseases including hypertension, diabetes, intestinal parasitizes, renal and cutaneous diseases. Bark is used as diuretic in Gabon and, fresh leaves are also used as food in Nigeria (Oteng-Gyang and Mbachu, 1987; Kimpouni, 1999; Okafor, 1999; Sarah, 1999).

Pterocarpus soyauxii contains various compounds such as biflavonoids (santalin A, santarubins A and B), isoflavonoids (pterocarpin, formononetin and prunetin), an isoflavone quinone (claussequinone), isoflavanes (vestitol and mucronulatol), tannins, ascorbic acid, glucosides, triterpenes, xanthones (Arnone et al., 1977; Banerjee and Mukherjee, 1981; Bezuidenhoudt et al., 1987;

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Kiec-Swierczynska et al., 2004; Surowiec et al., 2004). Other phenolic constituents have been isolated from *Pterocarpus marsupium* Roxb bark and *Pterocarpus santalinus* plant (Manickam et al., 1997; Kesari et al., 2004; Maurya et al., 2004).

There is a lack of experimental reports on the pharmacological activities of *Pterocarpus soyauxii* extracts. However, *Pterocarpus soyauxii* presents similarities in phytochemical composition with other species of the genus *Pterocarpus* which biological activities have been tested. Several components are known for their antidiabetic potential (Jung et al., 2006). Roxb-(−)-epicatechin, a water soluble benzopyran, stimulates insulin secretion (Hii and Howell, 1984). In addition, the biological activities of crude extracts of *Pterocarpus* species include hypoglycaemic and antidiabetic properties (*Pterocarpus marsupium* (Abesundara et al., 2004; Saxena and Vikram, 2004; Vats et al., 2004; Anandharajan et al., 2005; Dhanabal et al., 2006), *Pterocarpus santalinus* (Nagaraju et al., 1991; Kameswara Rao et al., 2001)) and also, cardiotonic activity (*Pterocarpus marsupium* (Mohire et al., 2007)), antimicrobial activity (*Pterocarpus santalinus* (Narayan et al., 2007); *Pterocarpus angolensis* (Steenkamp et al., 2004; Sami et al., 2009); *Pterocarpus indicus* (Ragasa et al., 2005)), antiulcerogenic activity (*Pterocarpus santalinus* (Narayan et al., 2007)). Methanolic extracts of *Pterocarpus santalinus* have also been reported to induce apoptosis of cancer HeLa cells (Kwon et al., 2006).

Despite knowledge of biological activities of the *Pterocarpus* genus, toxicological studies are very few. *Pterocarpus soyauxii* wood dust is known to cause skin irritation, allergic contact dermatitis and sensitization (Kiec-Swierczynska et al., 2004). Surprisingly, although its oral use is widespread, the oral toxicity of *Pterocarpus soyauxii* has not been studied.

Therefore, acute and sub-chronic toxicological studies were conducted in mice and rats, respectively, to evaluate the safety of the oral administration of an aqueous stem bark extract of *Pterocarpus soyauxii*.

2. Materials and methods

2.1. List of abbreviations

Alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), malondialdehyde (MDA), catalase (CAT), body weight (BW).

2.2. Plant material and preparation of the aqueous extract

Pterocarpus soyauxii (Papilionaceae) barks were collected in March 2002 in Nkolbibanda village (Central province, Cameroon) by Dr. L. Zapfack (Botany Department, University of Yaounde 1). The plant was identified at the National Herbarium of Yaoundé where a voucher specimen was deposited (No.: HNC/2427). The stem barks were dried at room temperature and ground into powder. Dry powder (200 g) was macerated in 2 l of boiling distilled water for 10 min and then kept 24 h at room temperature. The resulting aqueous extract was filtered. The filtrate was concentrated in a drying-room at 40 °C for 24 h, producing 24.10 g of red well-dried residue (w/w yield: 12.05%). The extract was stored at −20 °C.

2.3. Phytochemical screening

To determine the chemical constituents, qualitative phytochemical screening of the aqueous stem bark extract of *Pterocarpus soyauxii* was carried out following standard procedures routinely used in the laboratory (Tchamadeu et al., 2010) and revealed alkaloids (Meyer and Dragendoff's test), tannins ($FeCl_3$ test), saponins (frothing test), lipids (Wattman paper test), flavonoids (Schinoda's test), glycosides and polyoses (NaCl, and Fehling's solutions A and B), anthraquinones (ether–chloroform and NaOH), phenols ($FeCl_3$), polyphenols ($K_3Fe(CN)_6$), terpenoids (Liberman Burchard's test).

2.4. Animals

BALB/c mice and Wistar rats, raised in the animal core facility of the Faculty of Science, University of Yaoundé 1, were used. Animals were housed in colony cages (5 rats or mice per cage), under standard laboratory conditions (ventilated room, 24 °C, 75% humidity, 12 h light/dark cycle) and had free access to standard commercial diet and tap water. All animal experiments were conducted in accordance with the internationally accepted principles for laboratory animal use and care as described in the European Community guidelines (Official Journal of European Union L197 vol. 50, July 2007).

2.5. Acute oral toxicity study in mice

Healthy BALB/c mice (3-month old, 19–30 g BW) were randomly assigned to each of six groups of 10 mice (5 females and 5 males). Mice were fasted overnight (12 h) with free access to water prior to administration of single doses (0.0, 2.5, 5.0, 7.5, 10.0 and 12.5 g/kg) of the extract dissolved in distilled water. Treatment was given by gavage of 1 ml/100 g rat BW or of 0.5 ml/20 g mouse BW. The general behaviour of the mice was continuously monitored for 4 h after the treatment, intermittently during a 24-h period (Twaij et al., 1983), and thereafter daily up to 7 days. The LD_{50} was determined as previously described (Molle, 1986).

2.6. Sub-chronic oral toxicity study in rats

Healthy Wistar rats (3-month old, 145–265 g BW) were randomly assigned to each of four groups of 10 rats (5 females and 5 males). The extract, dissolved in distilled water, was administered by daily gavage for 42 days, to groups I to IV (doses of 0, 150, 300 and 600 mg/kg, respectively). The animals were observed for signs of toxicity and mortality throughout the experimental period. The BW, water and food consumption were recorded weekly. At the end of the 42-day experiment, the animals, fasted for 12 h, were sacrificed by decapitation under anaesthesia (thiopental 50 mg/kg). Blood was collected into two tubes: tube 1 containing EDTA was processed immediately for haematological parameters; tube 2 without additive was centrifuged at 3000 × *g* at 4 °C for 10 min to obtain serum (stored at −20 °C until analysis). The organs (kidneys, liver, lungs, heart, testes and glands annexes, ovaries, spleen and pancreas) were weighted. Organ samples (kidney, pancreas, lung and liver) were either fixed in 10% formalin for histopathological examination or stored at −20 °C until analysis of tissue total proteins, MDA, glutathione and catalase activities.

2.6.1. Blood analysis

Blood glucose was determined on days 0 and 42, using test strips (Accuchek, Roche Diagnostics), on 20 μl caudal vein blood samples obtained from fasted rats. Whole blood cell (WBC), red blood cell (RBC), leukocyte and platelet counts, hematocrit, haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), were determined as previously described (Tan et al., 2008), using an automatic analyser (System H1, Bayer Diagnostics). The serum was analysed as previously described (Tchamadeu et al., 2010) for total protein, cholesterol, creatinine, triglycerides and (ALT/AST) activities, using specific commercial diagnostic kits (Fortress Diagnostics, London, UK). Glutathione level was determined enzymatically (Ellman, 1959). The optical density at the appropriate wavelength was measured using a spectrophotometer (UV spectrophotometer Hitachi).